Spleen-on-a-Chip Sepsis Therapeutic Device

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Disclaimer: The views expressed are those of the author and do not reflect the official policy or position of the Department of Defense or the U.S. Government.

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Team Overview

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Spleen-on-a-Chip Concept, Microfluidics, medicine, physiology

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Biomaterials Protein Engineering, MAGE

J. Aizenberg
Adaptive architecture, SLIPS

M. Super
Protein Engineering, Wyss-MBL

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Microfluidics, magnetics

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SLIPS, hematology

D.Leslie
Microfluidics, chemistry

T-S Wang
SLIPS

M. Cartwright
Protein Engineering

A. Watters
Protein Engineering

J. Kang
Microfluidics

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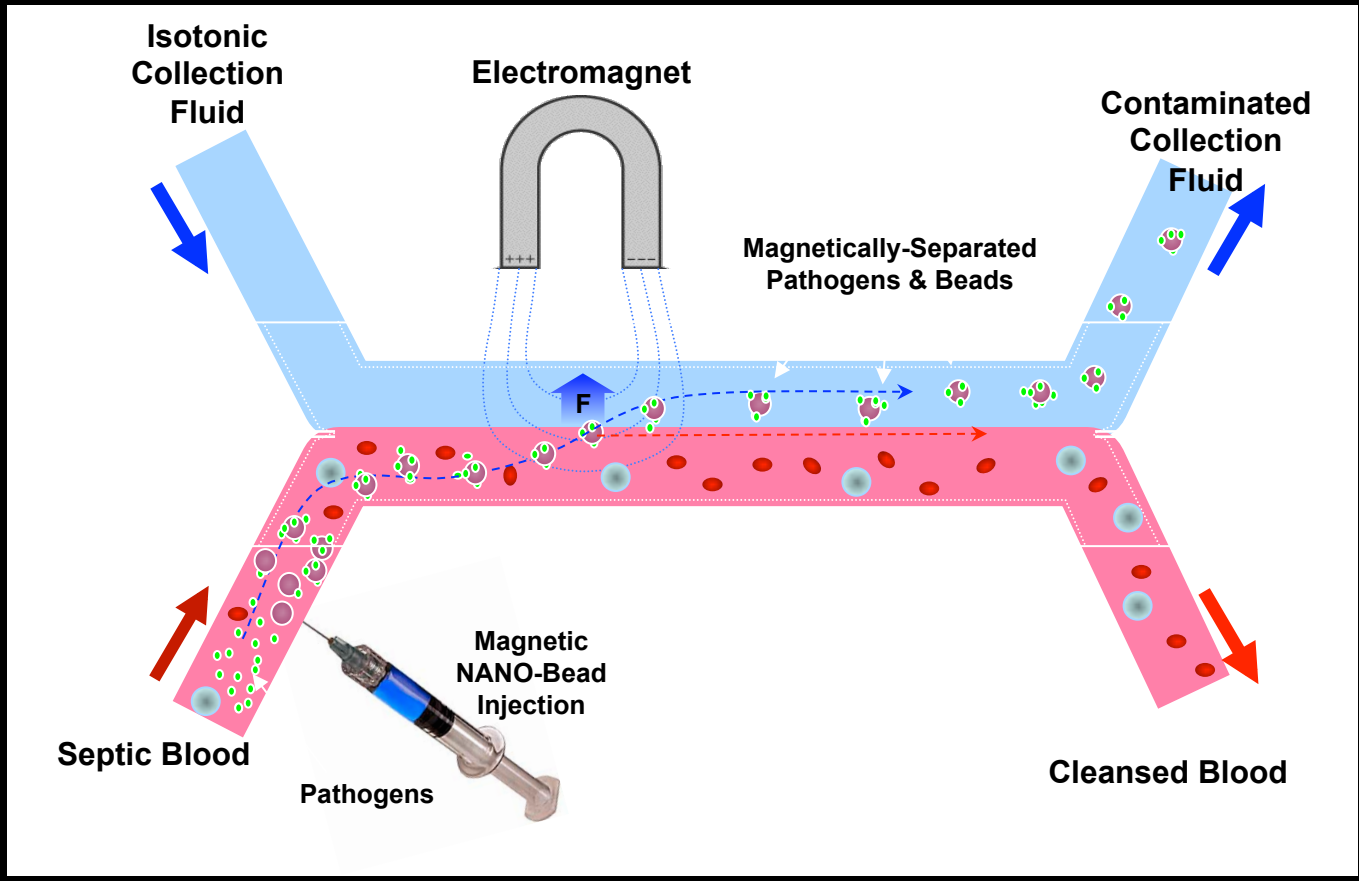
Technical Approach

MICROMAGNETIC-MICROFLUIDIC CELL SEPARATION CONCEPT

An extracorporeal microdevice that functions like an "Artificial Spleen" for Sepsis Therapy



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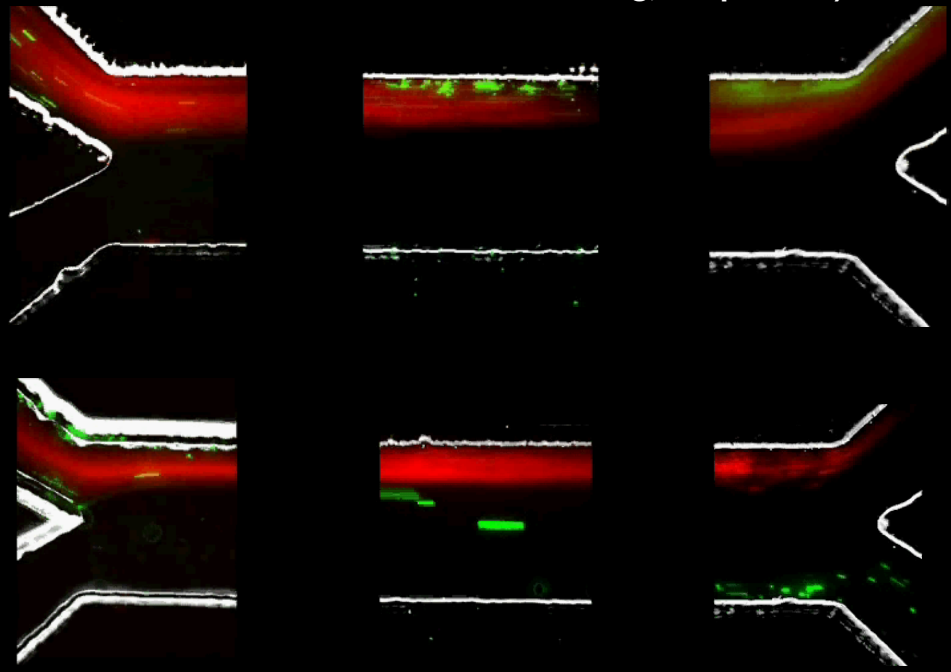
Magnetic Separation in Flow

(Work of Chong Yung, R. Cooper, Childrens Hospital & Wyss Institute; Jason Fiering, Draper Lab)

- Magnet

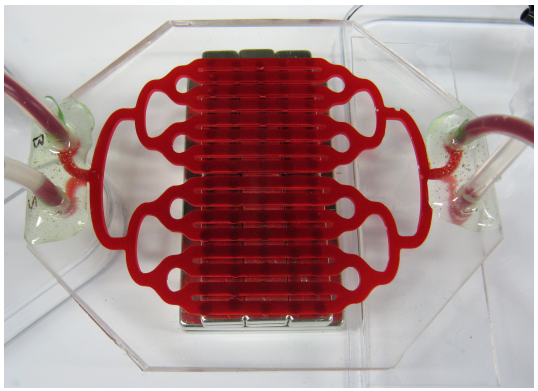
+ Magnet

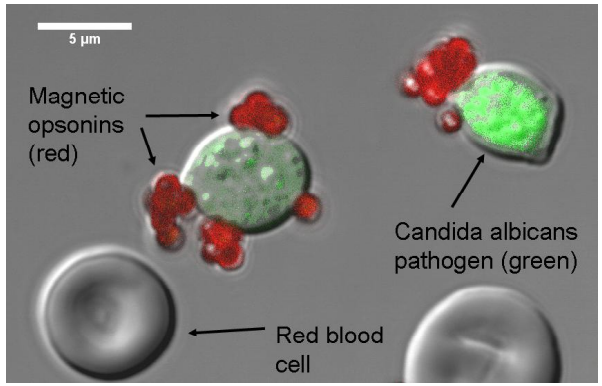


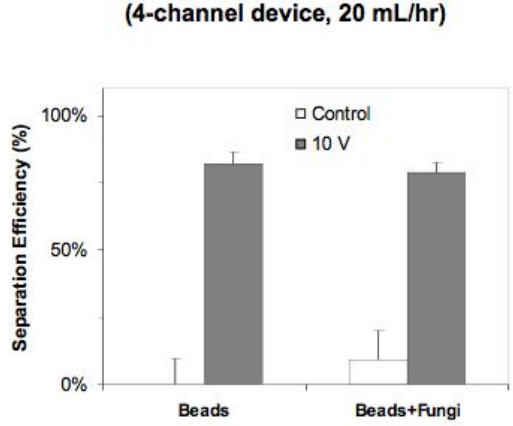
(Xia et al, *Biomed. Microdev.*, 2006; Yung et al., *Lab on a Chip* 2009)

C. *albicans* Fungi in Whole Human Blood

(with funding from CIMIT & Wyss)







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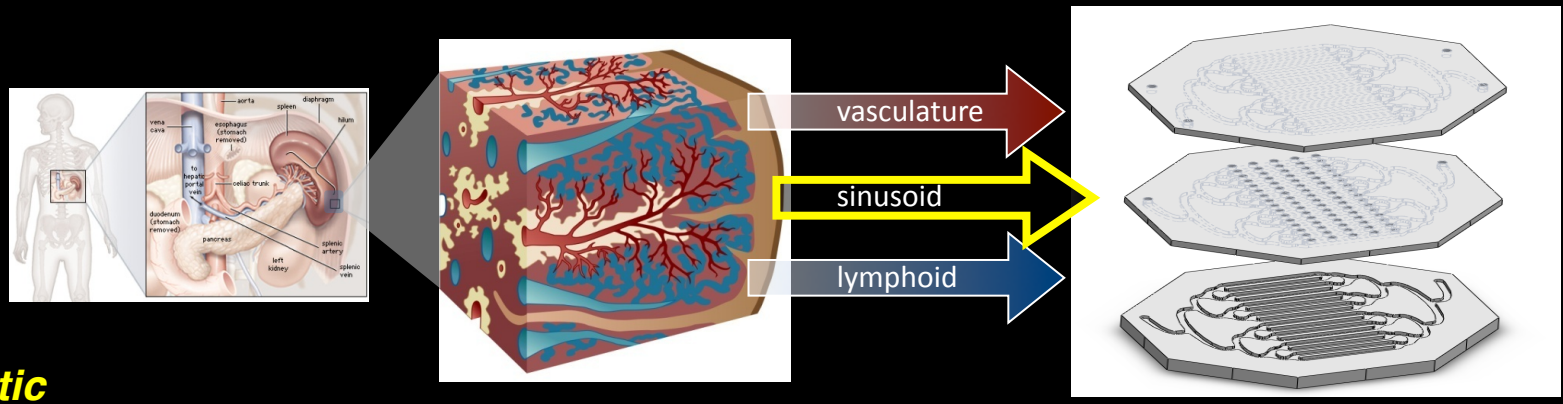
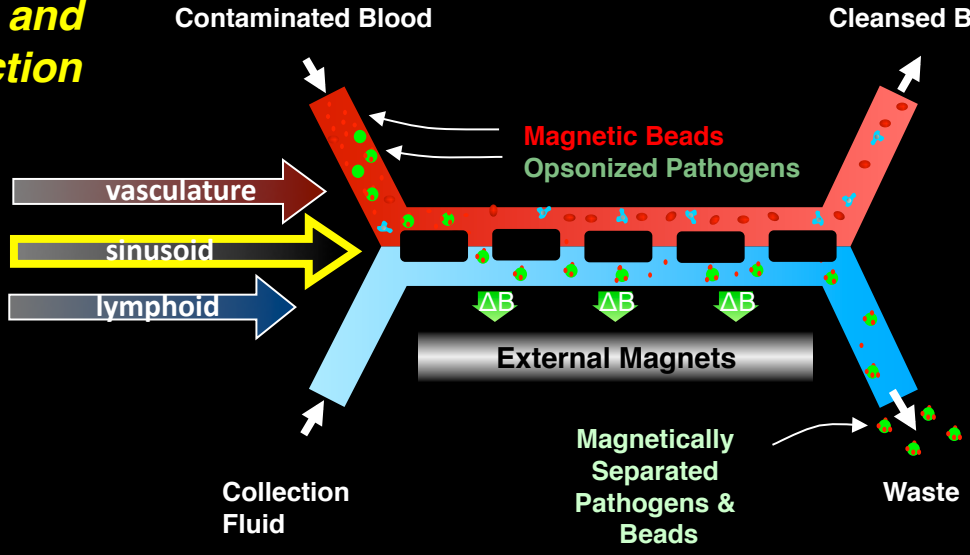
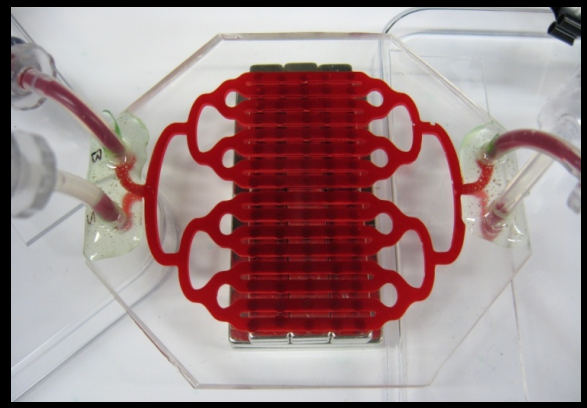
Improved Bioinspired Microfluidic Design



Biomimetic translation of form and function





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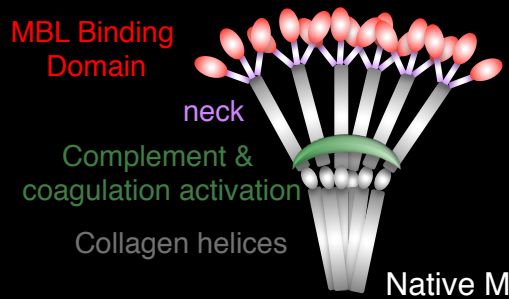
Spleen-on-a-Chip Sepsis DLT Device

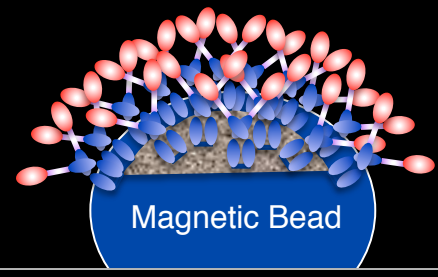
Current DARPA grant (MTO: Tim Broderick)

1) Mannose Binding Lectin (MBL) Engineering & Magnetic Opsonins



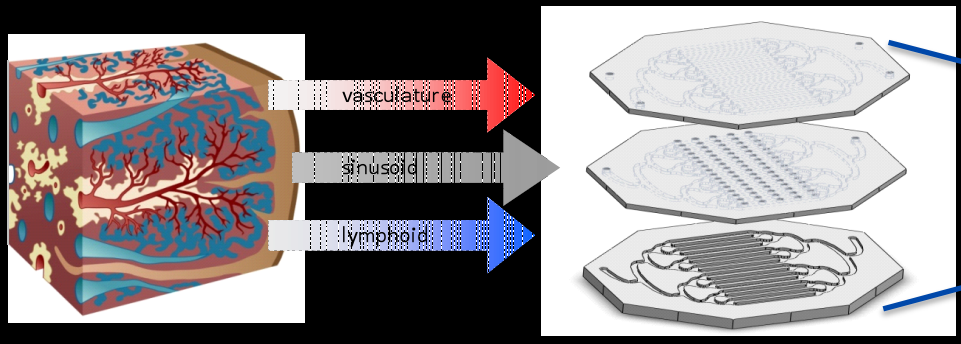
Native MBL (650kDa)

MBL-fp1 (90kDa)

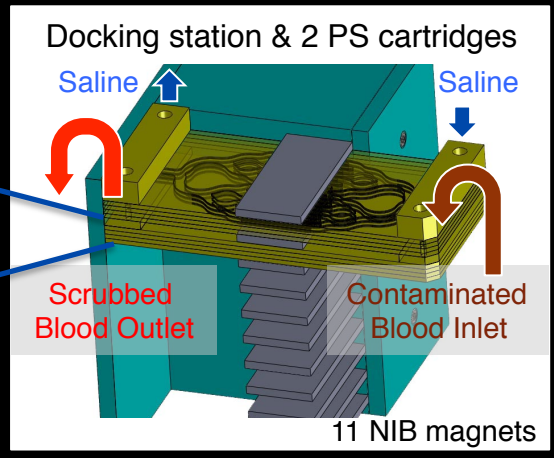


SUCCESS = Remove >90% of unknown pathogens from flowing blood within 24 hrs without coagulation

2) Pathogen removal with Spleen-on-a-Chip Device



Spleen **Bio-Spleen**



Multiplexed Bio-Spleen DLT Device

3) Accomplish separations without anticoagulants

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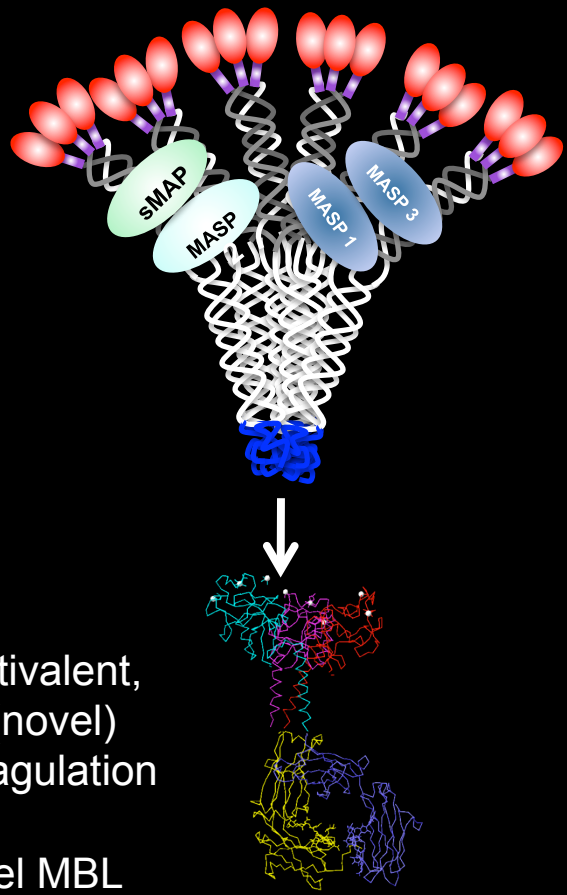
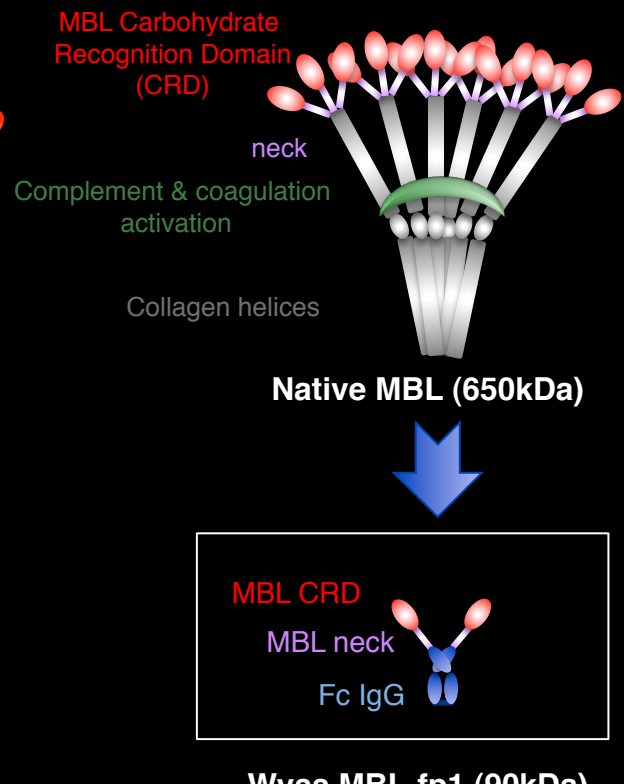
Protein Engineering of MBL

Native

1. Multimerization drives multivalent microbe recognition (18mer)
2. But: Multimerization drives complement & coagulation activation w/ MASP proteins
3. Complicated, carbohydrate based affinity purification for high mw MBL activity





Wyss MBL (MBL-fp1)

1. Magnetic beads provide multivalent, oriented, reversible binding (novel)
2. MASP Complement and Coagulation activity removed
3. Fc-linkage provides high level MBL expression, proper folding and easy purification (low cost)

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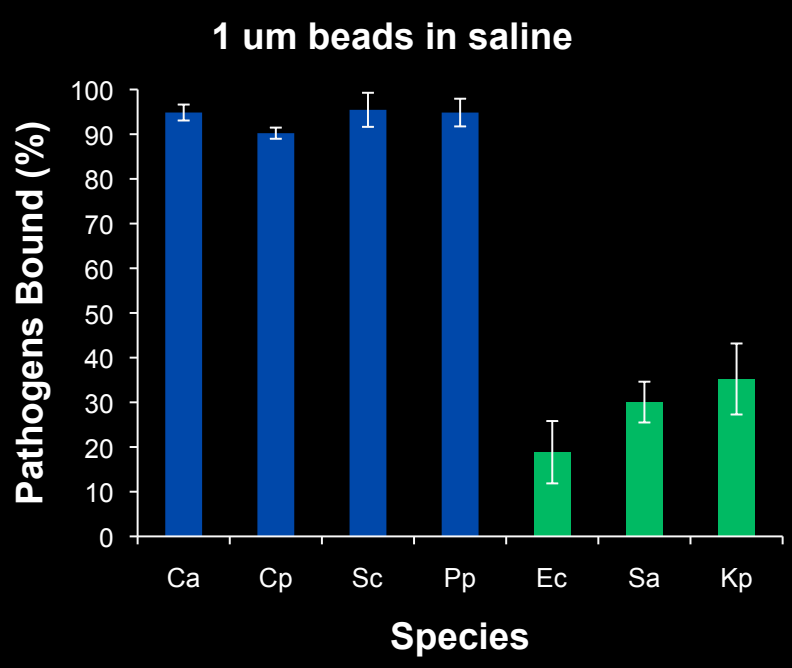
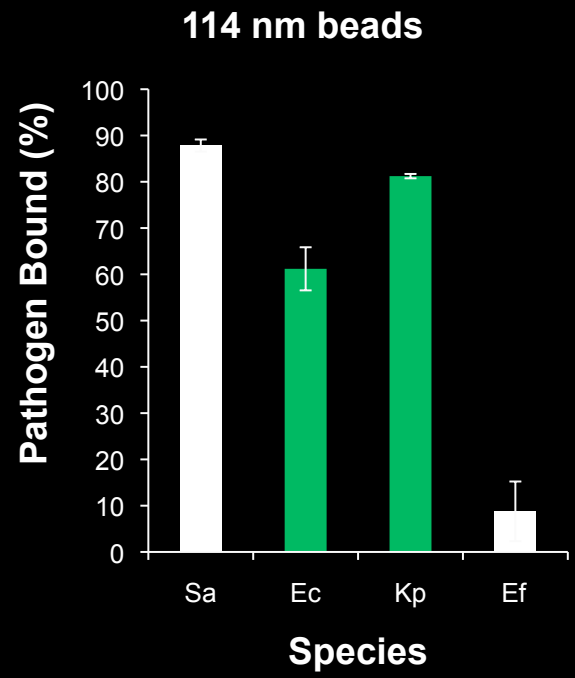
MBL-Magnetic Bead Binding

(8 Species & 3 Microbial Classes)





Fungi: blue

Ca: Candida albicans

Cp: Candida parasilosis

Sc: Saccharmoyces cerevisiae

Pp: Pichia pastoris

Bacteria : green (G-) or black (G+)

Sa: Staphylococcus aureus (G+)

Ec: Escherichia coli (G-)

Kp: Klebsiella pneumoniae (G-)

Ef: Enterococcus faecalis (G+)

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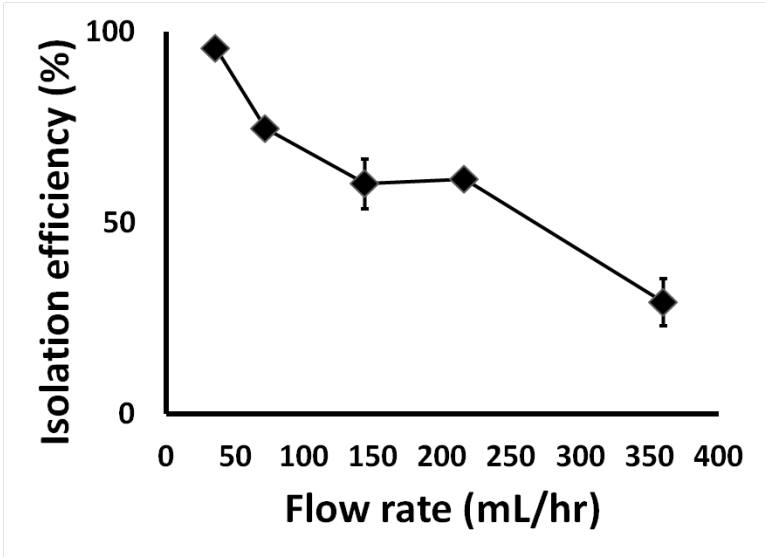
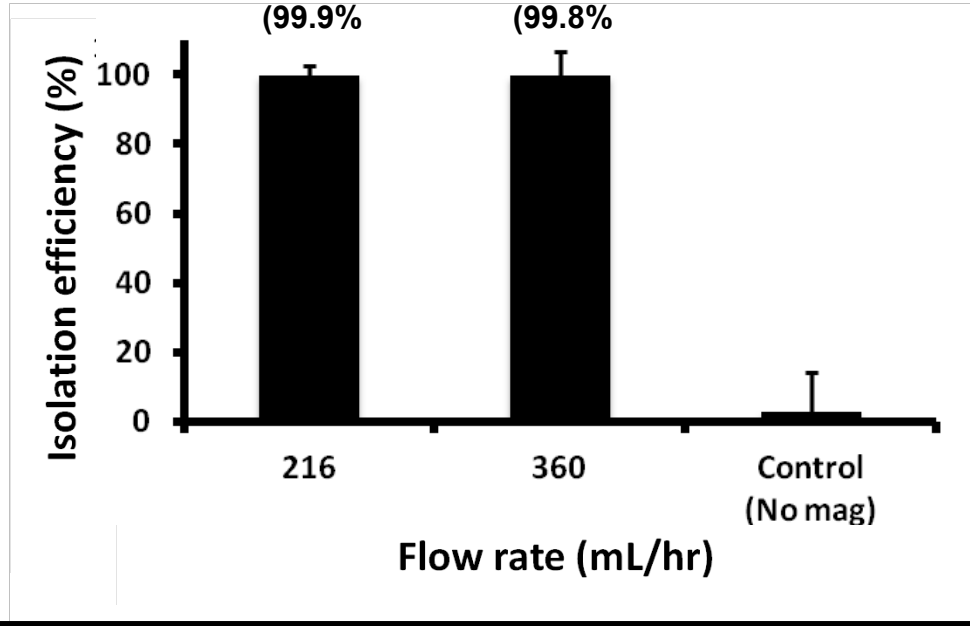
Clearance of Pathogens (*C. albicans*)
From Anticoagulated Whole Human Blood

- Using magnetic microbeads coated with MBL-fp1
- Beads bound to pathogen before being introduced into blood
- only 1 pass through the Polysulfone microfluidic device



Dec. 2011 Results



Feb. 2012 Results
(improved microsystem design)

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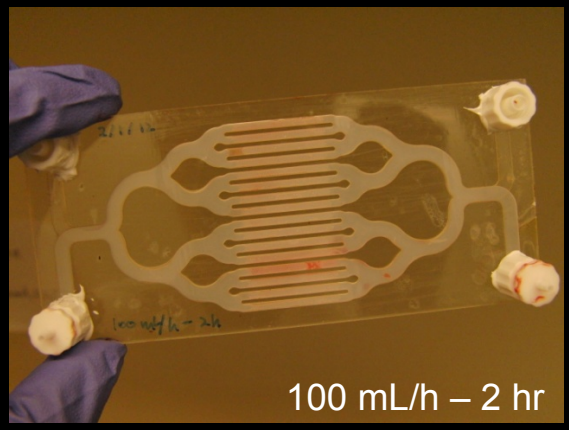
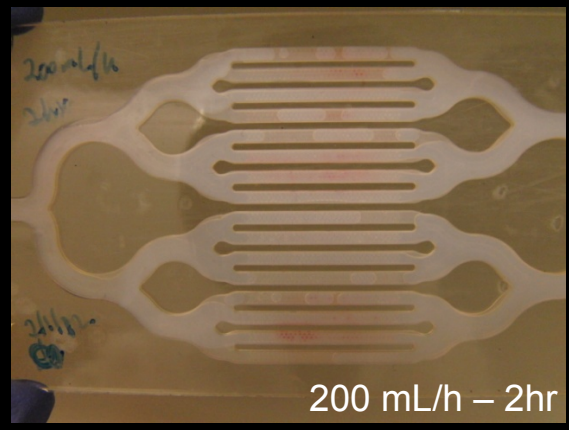
Anticoagulation Challenge: Current High Flow Rate DLT Device

- Heparinized human blood (20 mL)
- Animal model scale polysulfone DLT device (taped)
- Flow rate: 100 mL/h and 200 mL/h for 2 hours
- No clots observed in the DLT device
- No coagulation detected using Thrombin-AntiThrombin (TAT) assay
- $\tau \leq \sim 1$ dyne/cm^2





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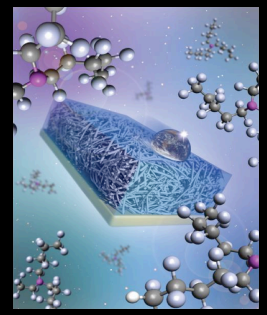
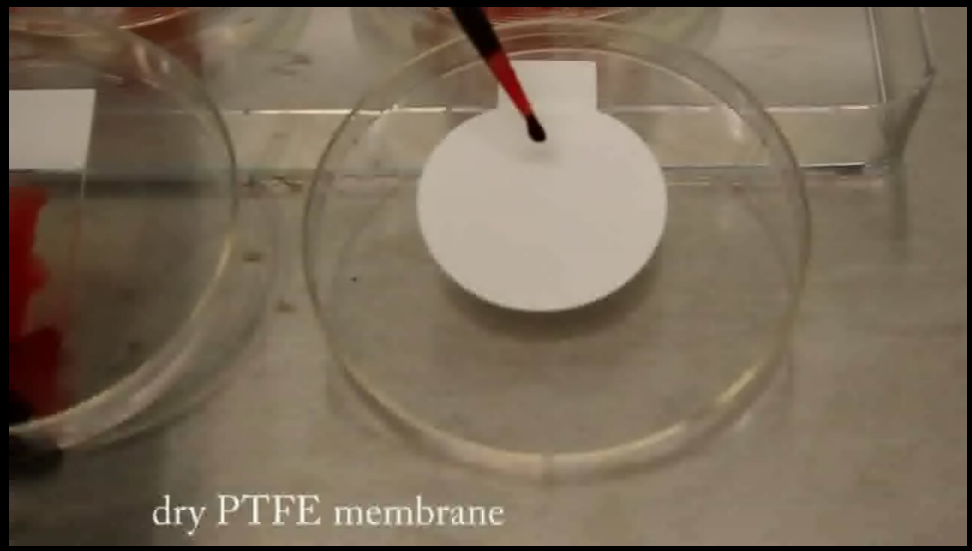
Slippery Liquid-Infused Porous Surfaces (SLIPS)

(Joanna Aizeberg Team: Wong et al., *Nature* 2011)



Fresh Whole Human Blood (*NON-HEPARINIZED*)



PTFE	SLIPS	PTFE	SLIPS
Static for 1 hr		Shaking for 1 hr	

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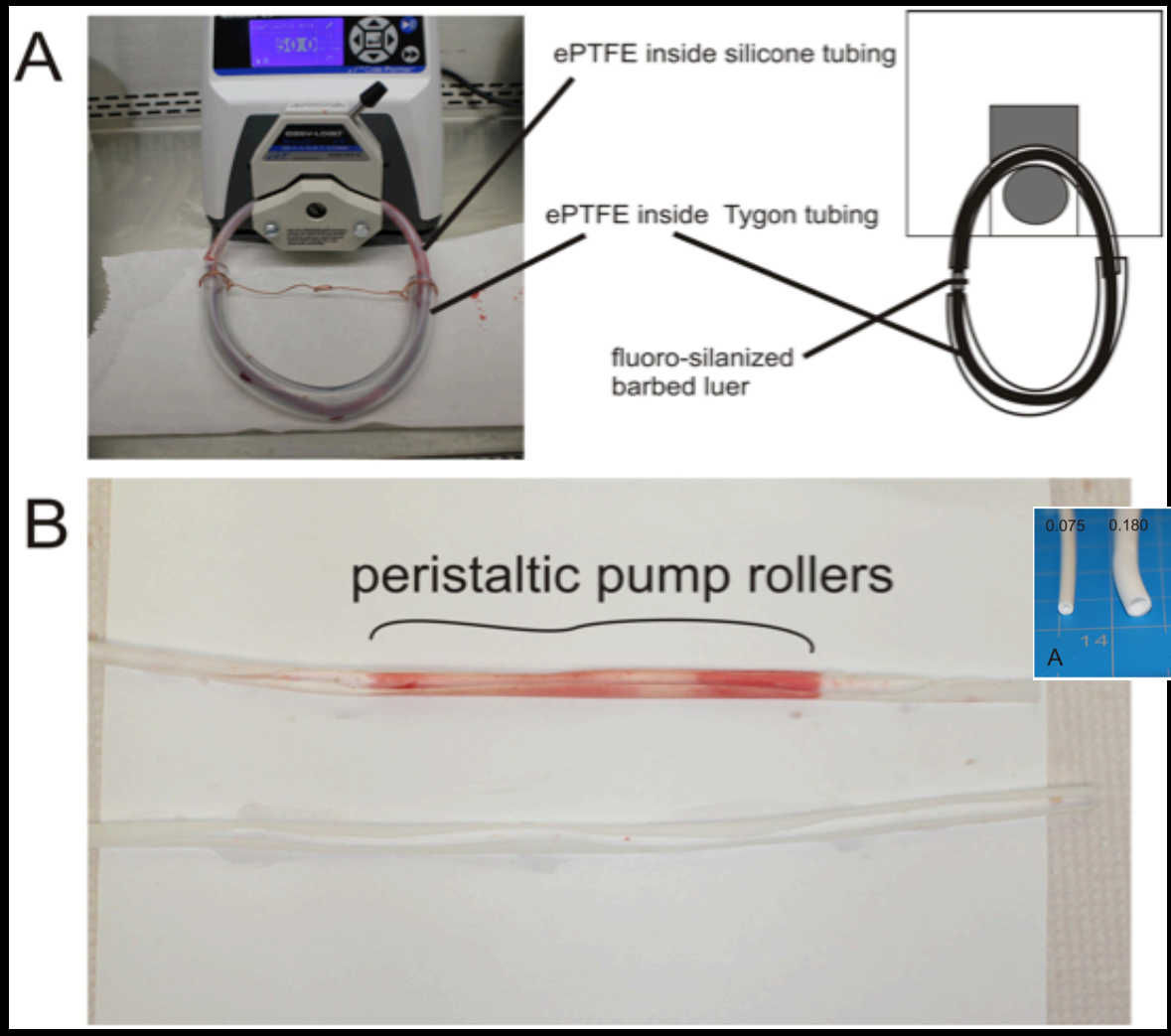
Flowed NON-anticoagulated whole human blood (diluted 1:1 with saline) @ 3,000 mL/hr for 20 min without clotting



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Transition Opportunities

Spleen on a chip – therapeutic device for pathogen removal that also can be sued for cleansing blood of other "contaminants"

1. Toxins
2. Inflammatory Mediators
3. Cancer cells

MBL – the MBL-fp1 has a broad range of potential uses in both the medical and veterinary fields

1. Therapeutic applications to clear pathogens from blood or other substrates
2. Diagnostic use to capture pathogens from various substrates, including blood, urine, food, water, etc and concentrate for further analysis
3. Diagnostic to ID pathogens in these samples

SLIPs – there are broad uses for the SLIPS technology in medical devices

1. Catheters, cannulae, shunts, vascular grafts

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Schedule and Major Tasks

YEAR 1 TASKS:

- **Task 2A:** 100 mL/hr microfluidic blood flow for ≥ 2 hours without platelet activation or clotting
- **Task 2B:** 100 mL/hr microfluidic blood flow for ≥ 2 hours without platelet activation or clotting using *anticoagulant surfaces*
- **Task 3A:** Stably express and produce > 500 mg of MBL-fp1; establish functional opsonin display phage library; develop screening assays for binding to novel pathogens
- **Task 3B:** Isolate > 50% of pathogens from 2 microbial classes from whole blood using a wide spectrum opsonin
- **Task 3C:** Develop effective extracorporeal blood perfusion technique using a biomimetic spleen DLT device in rats; establish and characterize the rat sepsis model

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Technical Progress to Date

- **Flowed anticoagulated whole human blood at 36-360 ml/hr without clotting** through microfluidic DLT device
- **Separated > 99.8% of fungal pathogens at 360 ml/hr flow rate in whole human blood** using DLT microfluidics device with magnetic MBL-fp1 opsonins.
- ***Flowed NON-anticoagulated whole human blood (diluted 1:1 with saline) at 3,000 ml/hr for 20 min without clotting***
- **Purified 100 mg of MBL-fp1** from mammalian cells transfected with genetically engineered MBL-fp1 & initiated Phase Display studies
- **Separated 8 different pathogens from 3 microbial classes (fungi, gram + & - bacteria) from human blood**
- **Establishing the rat sepsis model**

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Intellectual Property

- **We have filed a broad portfolio of intellectual property (> 20 patent applications) relating to the Spleen-on-a Chip Project**

- **Spleen-on-a-Chip Blood Cleansing Device**
  - Coverage of device components and methods of use

- **MBL–fp1 Opsonin**
  - Composition of matter on MBL forms
  - Use of MBL in blood cleansing
  - Additional uses of MBL, including for diagnostic and therapeutic indications relating to pathogen ID and treatment

- **SLIPS Anticoagulant Surfaces**
  - Methods to treat various surfaces to impart slippery characteristics
  - Uses of SLIPS materials to repel various biological materials, including blood and pathogens

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